REMARKS

Reconsideration and allowance of the subject application is solicited.

We first thank the Examiner for courtesies extended to applicant's representative during the telephone interview on June 14, 2010. The substance of the interview is summarized here.

In the May 11, 2010 Office Action, all of the previous rejections and objections were withdrawn. The Examiner lodged two new art rejections, citing one new reference, Gasson et al. Specifically, claim 1 is rejected under 35 U.S.C. §103(a) as obvious over Uraji et al. in view of Blum-Oehler et al (WO 99/44134, cited in ISR) in view of Trevors et al., and further in view of Gasson et al. Claims 2-6 are rejected under 35 U.S.C. §103(a) as obvious over Uraji et al. in view of Blum-Oehler et al (WO 99/44134, cited in ISR) in view of Trevors et al., and Gasson, and further in view of Alexeyev et al.

The Examiner cites Uraji for teaching a method to cure a single plasmid from a gram negative bacteria – not the DSM 6601 strain or any E. coli strain, or the pMut plasmids, or curing more than one plasmid. Blum-Oehler is cited for the disclosure of Nissle 1917 (DSM 6601), but the Examiner acknowledges that this reference does not teach a plasmid-free clone of DSM 6601. The Examiner argues that Blum-Oehler teaches that the Nissle 1917 strain has two plasmids (pMut1 and pMut2) that are cryptic and without benefit to the host. The Examiner cites Trevors et al. for describing curing methods for removing bacterial plasmids. Alexeyev is cited for its description of a tetracycline resistance cassette in a plasmid. Responding to one of our arguments in the last Response we filed, that none of the references teach curing more than one plasmid, the Examiner has cited a new reference – Gasson et al. – for teaching the curing of five plasmids from a Streptococcus strain.

To find our claims obvious, the Examiner needed to cite a string of four references (five for the method claims) – even so, **no reference specifically teaches the curing of DSM 6601 or the desire to cure it of both pMut1 and pMut2**. The Gasson reference is cited for motivation to cure two or more plasmids, and even that reference deals with an entirely different strain (Streptococcus), and addresses a different problem, and solves its problem using a different curing process altogether.

Gasson has been cited from a retrospective point of view – that is, to fill in a "gap" in the string of cited art, to show that a plasmid can be cured from a host. In Gasson's disclosure, a Streptococcus strain is cured of five plasmids.

Curing strains of Streptococcus having a large complement of plasmids requires several obstacles to be overcome. Difficulties addressed by Gasson include the assignment of suspected plasmid-encoded phenotypes to individual molecules, the complicated analysis of plasmid transfer experiments, and the equally complicated analysis of the molecular study of individual plasmids from the complement (as also noted by the Examiner). The focus of the Gasson article is how to deal with these particular issues. The curing process itself is conducted as a protoplastic-promoted curing, which means that plasmid-loss occurs during the early stages of protoplast regeneration.

In the situation of the E. coli DSM 6601 of our invention, the pMut1 and pMut2 plasmids are both cryptic. There was no issue regarding which plasmid to cure. And of course, the method our inventors used for double-curing is completely different from the Gasson method. As we noted in a previous response – and as is well accepted in this art – not every curing method is suitable for curing each bacterial species. Clearly, the method employed by Gasson is nearly irrelevant to the method our inventors adapted, and would not have been effective to identify or address the problem encountered by our method. The only parallel between our invention and Gasson is that a bacterial species is cured of more than one plasmid. However, the differences between our technology and Gasson's – different bacterial species, different problems encountered and overcome, different methods of curing – are simply too significant to be minimized or overlooked, which is what would be necessary to have this reference fill in the "gap" of the other cited art. For the purposes of our particular invention, Gasson would not have been a reference someone skilled in this art would have looked to for direction to cure DSM 6601 of the pMut1 and pMut2 plasmids.

The primary reference Uraji is itself quite irrelevant to our claims on many points, as is evident by the string of secondary references cited to make up for its deficiencies.

As noted in our past response, Uraji teaches carefully that its curing techiques "should also be applicable to other types of plasmids in *Agrobacterium* groups". (See Abstract of Uraji et al.). From the standpoint of someone having ordinary skill in this art, *Agrobacterium* is neither genetically, physiologically or microecologically comparable to the probiotic, non-pathogenic E. coli strain DSM 6601, which is used in humans to treat gastrointestinal dysfunctions and diseases. *Agrobacterium* can only infect and colonize plants; *E. coli* is a bacterium that populates the human and animal intestine. *Agrobacterium* and *E. coli* are two completely different organisms, and the removal or even desirability of removal of the plasmids would not be viewed by someone having ordinary skill in this art as being equivalent or even suggestive of each other. By their affirmative suggestion that their curing methods should be applicable to other types of plasmids in the *Agrobacterium* species, Uraji's own authors obviously are not suggesting to extrapolate their technique for use in curing plasmids in other bacterial species. Someone having ordinary skill in this art would likewise not have been reasonably expected Uraji's method to be useful for curing *E. coli* or other types of non-*Agrobacterium* species.

Further, Uraji teaches the introduction of the sacB gene on only one plasmid; the second plasmid does not have sacB gene. In the Office Action at page 6, The Examiner argues that "if two plasmids are being introduced in to the cell, it would follow that both plasmids would contain the sacB gene in order to cure the cell." This is an interpretation of the Uraji reference that may or may not be correct. With all due respect, the Examiner is apparently not sure what Uraji teaches – how can someone having ordinary skill be assumed to reach the same conclusion, AND be reasonably lead to apply that to the teachings of the other references. Furthermore, the introduction of two sacB genes into the pMut1 and pMut2 plasmids is an affirmative requirement in our claims 2-6. Uraji, or any of the other cited references, do not teach or suggest the deliberate and separate introduction of two sacB genes, one into each of the pMut1 and pMut2 plasmids. The USPTO examination guidelines provide that, where an element from a secondary reference is substituted into the teaching from the primary reference, the primary reference should at least suggest that the modification could be successful (Exam

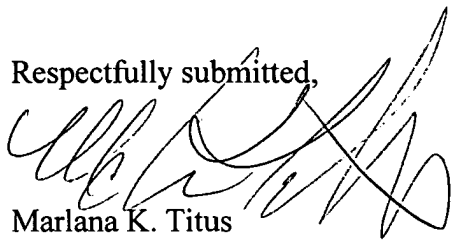
Guideline B). Here, Uraji as a primary reference falls short to help establish a prima facie case of obviousness.

Regarding the Blum-Oehler reference, the Examiner cites it on the basis that it teaches that DSM 6601 is useful as a probiotic drug against intestinal disorders, and its method is a much safer way of curing, and further that Blum-Oehler states that the pMut1 and pMut2 plasmids are cryptic and have no apparent benefit to the host. We agree that Blum-Oehler et al. describes the cryptic plasmids pMUT1 and pMUT2 of E. coli strain Nissle 1917 (syn. DSM 6601). In particular, this reference describes a PCR-based method for the specific detection of E. coli DSM 6601, which can be used to specifically detect E. coli DSM 6601 in the fecal flora of patients in the presence of other E. coli strains. The DNA sequences of the plasmids are described and their use for diagnostic and biotechnological purposes. However, as noted by the Examiner, Blum-Oehler does not suggest a plasmid-free DSM 6601 strain. At best, Blum-Oehler represents the problem in the art solved by our plasmid-free strains – it presents absolutely no solution to its own problem.

In order to combine these references to make the current claims obvious, hindsight reconstruction is necessary using the applicants' own disclosure as a basis. The primary reference is too irrelevant, and the secondary references are too disconnected from our invention. It is impermissible to use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention. (In re Fine, 837, F.2d 1071 (Fed. Cir. 1988); In re Fritch 972 F.2d 1260 (Fed. Cir. 1992). Using the inventor's success as evidence that one of ordinary skill in the art would have reasonably expected success represents an impermissible use of hindsight. Life Technologies, Inc. v. Clontech Laboratories, Inc., 224 F.3d 1320 (Fed. Cir. 2000).

In summary, all of the Examiner's outstanding rejections and objections have been addressed, and the application is believed to be in allowable form. Notice to that effect is earnestly solicited. If the Examiner has any questions or would like to make suggestions as to claim language, she is encouraged to contact Marlana K. Titus at (301) 977-7227.

Respectfully submitted,



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